BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PHYSIOLOGIA PLANTARUM 93: 365–374. 1995
Printed in Denmark – all rights reserved

ISSN 0031-9317

Minireview

The plant cell cycle

Dennis Francis and Nigel G. Halford

Francis, D. and Halford, N.G. 1995. The plant cell cycle. – Physiol. Plant. 93: 365–374.

The first aim of this paper is to review recent progress in identifying genes in plants homologous to *cell division cycle* (*cdc*) genes of fission yeast. In the latter, *cdc* genes are well-characterised. Arguably, most is known about *cdc2* which encodes a 34 kDa protein kinase ($p34^{cdc2}$) that functions at the G2-M and G1-S transition points of the cell cycle. At G2-M, the $p34^{cdc2}$ protein kinase is regulated by a number of gene products that function in independent regulatory pathways. The *cdc2* kinase is switched on by a phosphatase encoded by *cdc25*, and switched off by a protein kinase encoded by *wee1*. p34 Must also bind with a cyclin protein to form maturation promoting factor before exhibiting protein kinase activity. In plants, homologues to $p34^{cdc2}$ have been identified in pea, wheat, *Arabidopsis*, alfalfa, maize and *Chlamydomonas*. They all exhibit the PSTAIRE motif, an absolutely conserved amino acid sequence in all functional homologues sequenced so far. As in animals, some plant species contain more than one *cdc2* protein kinase gene, but in contrast to animals where one functions at G2-M and the other (*CDK2* in humans and *Eg1* in *Xenopus*) at G1-S, it is still unclear whether there are functional differences between the plant $p34^{cdc2}$ protein kinases. Again, whereas in animals cyclins are well characterised on the basis of sequence analysis, into class A, class B (G2-M) and CLN (G1 cyclins), cyclins isolated from several plant species cannot be so clearly characterised. The differences between plant and animal homologues to $p34^{cdc2}$ and cyclins raises the possibility that some of the regulatory controls of the plant genes may be different from those of their animal counterparts.
The second aim of the paper is to review how planes of cell division and cell size are regulated at the molecular level. We focus on reports showing that $p34^{cdc2}$ binds to the preprophase band (ppb) in late G2 of the cell cycle. The binding of $p34^{cdc2}$ to ppbs may be important in regulating changes in directional growth but, more importantly, there is a requirement to understand what controls the positioning of ppbs. Thus, we highlight work resolving proteins such as the microtubule associated proteins (MAPs) and those mitogen activated protein kinases (MAP kinases), which act on, or bind to, mitotic microtubules. Plant homologues to MAP kinases have been identified in alfalfa. Finally, some consideration is given to cell size at division and how alterations in cell size can alter plant development. Transgenic tobacco plants expressing the fission yeast gene, *cdc25*, exhibited various perturbations of development and a reduced cell size at division. Hence, *cdc25* affected the cell cycle (and as a consequence, cell size at division) and *cdc25* expression was correlated with various alterations to development including precocious flowering and altered floral morphogenesis. Our view is that the cell cycle is a growth cycle in which a cell achieves an optimal size for division and that this size control has an important bearing on differentiation and development. Understanding how cell size is controlled, and how plant *cdc* genes are regulated, will be essential keys to 'the cell cycle locks', which when 'opened', will provide further clues about how the cell cycle is linked to plant development.

Key words – *cdc* Genes, cell cycle, cell size, cyclins, meristems.

D. Francis (corresponding author), School of Pure and Applied Biology, Univ. of Wales, P.O. Box 915, Cardiff CF1 3TL, UK, and N.G. Halford, Dept of Agricultural Sciences, Univ. of Bristol, Inst. of Arable Crops Research, Long Ashton Research Station, Bristol BS18 9AF, UK.

Received 15 September, 1994

Introduction

During the past 10 years, cell cycle research has led to exciting discoveries. In the 1980's, the vast array of mutants in budding yeast and fission yeast, blocked at various points in the cell cycle, were ripe for exploitation by molecular techniques. Complementation with wild type DNA restored normal growth and led to the subsequent identification of the defective *cell division cycle* (*cdc*) gene(s). In particular, *cdc2* in fission yeast was found to be essential for two check-points in the cell cycle, one in late G2 and the other in late G1 (Nurse 1990, Hayles and Nurse 1993). *cdc2* Encodes a 34 kDa threonine/serine protein kinase. In order to exhibit kinase activity, $p34^{cdc2}$ must bind to another protein, cyclin B (encoded by *cdc13* in fission yeast, Solomon et al. [1988] commenting on Booher and Beach 1988) to form maturation promotion factor (MPF; Gautier et al. 1990, Parker et al. 1991). In fission yeast, phosphorylation of $p34^{cdc2}$ at Thr167 is also essential prior to binding to cyclin B; without MPF mitosis does not occur (Krek and Nigg 1991). Homologues to fission yeast $p34^{cdc2}$ have now been isolated from a range of unrelated organisms, including humans and higher plants (Lee and Nurse 1987, John et al. 1989, Elledge and Spotswood 1991).

Characterisation of the cyclin subunit of MPF came through independent biochemical and genetic analyses of the same protein/gene in different organisms. In fission yeast, cyclin B is a 56 kDa protein which is analogous to the 45 kDa subunit (cyclin B) of MPF in *Xenopus*; the other subunit is a 34 kDa protein which cross-reacts with a strongly conserved peptide of p34 (Labbé et al. 1989). Subsequently, numerous other papers have demonstrated the universality of MPF in various unrelated organisms, e.g. yeasts, *Xenopus*, *Drosophila*, gold-fish, man (Lee and Nurse 1987, Edgar and O'Farrell 1989, Richardson et al. 1990, Yamashita et al. 1992). MPF is at the bottom of a regulatory cascade involving numerous interacting proteins, many of which are protein kinases.

The transition between G2 and mitosis is best understood in fission yeast where a set of well-defined genes function (see Nurse 1990). Two independent regulatory pathways act as switch-on/switch-off mechanisms for $p34^{cdc2}$ kinase activity. These are:

nim1 (+) → wee1 (−) → cdc2
cdc25 (+) → cdc2

Wee1 encodes a protein kinase ($p107^{wee1}$) which phosphorylates $p34^{cdc2}$ (off signal) while *nim1* probably encodes a protein kinase which phosphorylates and inactivates *wee1*. *cdc25* Encodes a phosphatase ($p80^{cdc25}$) which dephosphorylates $p34^{cdc2}$ (on signal; Kumagai and Dunphy 1991).

Mutants of fission yeast which over-express *wee1* exhibit a larger-than-wild-type phenotype, whereas those that over-express *cdc25* or *nim1* exhibit a smaller-than-wild-type phenotype; the former delays entry of cells into division and, hence, they grow larger, whereas the latter divide prematurely (Russell and Nurse 1986, 1987a, b). In effect, the two pathways regulate cell size at division. A human homologue of *wee1* exists which phosphorylates *cdc2* exclusively on Tyr15 (McGowan and Russell 1993), whereas a *mik1* protein kinase can act interchangeably with p107 (Lundgren et al. 1991). Also, homologues to *cdc25* exist in a range of unrelated organisms including *Drosophila* (string) (Edgar and O'Farrell 1990) and humans (twine) (Strausfeld et al. 1991, Alphey et al. 1992).

What of the plant cell cycle? The application of molecular techniques to the plant cell cycle followed soon after the land-mark discoveries that homologues exist to *cdc2* in a range of unrelated organisms. The aim of this paper is to review the recently discovered plant cell division cycle genes and to provide a follow-on from earlier reviews of the plant cell cycle (see, for example, Davidson 1991, Francis 1992, Jacobs 1992). We shall examine plant *cdc* genes in relation to the proteins they encode, the proteins' catalytic or functional activity, and importantly, what we think the link is between them and plant development. The underlying controls which regulate the planes of cell division are central to deciphering the relationship between cell division and plant development. Hence, we also review progress in this important area. Nuclear DNA replication is excluded; the reader is referred to a recent detailed account of the regulation of S-phase in higher plants (Aves and Bryant 1993).

Abbreviations – MAP, microtubule associated protein; MAP kinase, mitogen activated protein kinase; MPF, maturation promotion factor; ppb, preprophase band.

Isolation of plant homologues to *cdc* genes

cdc2

As the network of interacting genes and proteins regulating the yeast cell cycle started to become clear, and homologues of some of the genes were identified in animals, plant molecular and cell biologists began to ask whether a similar system existed in plants. The fascinating answer to this question has far-reaching consequences because manipulation of such a system by genetic transformation could shed new light on the role of cell division in the control of plant development. The strategy adopted by many workers has been to isolate plant homologues of specific yeast *cdc* genes or to identify their gene products; this was first achieved immunologically with *cdc2*.

The first evidence that a $p34^{cdc2}$ homologue is present in plant cells was obtained by John et al. (1989) using three antibodies. The first was raised to the L13V-peptide (subsequently referred to as the PSTAIR peptide) with the sequence: Glu Gly Pro Ser Thr Ala Ile Arg Glu Ile Ser Leu Leu Lys Glu. This sequence is present in the fission yeast $p34^{cdc2}$ from amino acids Glu^{42} to Glu^{57} and is absolutely conserved in all the functional homologues sequenced so far. The other antibodies were raised to a

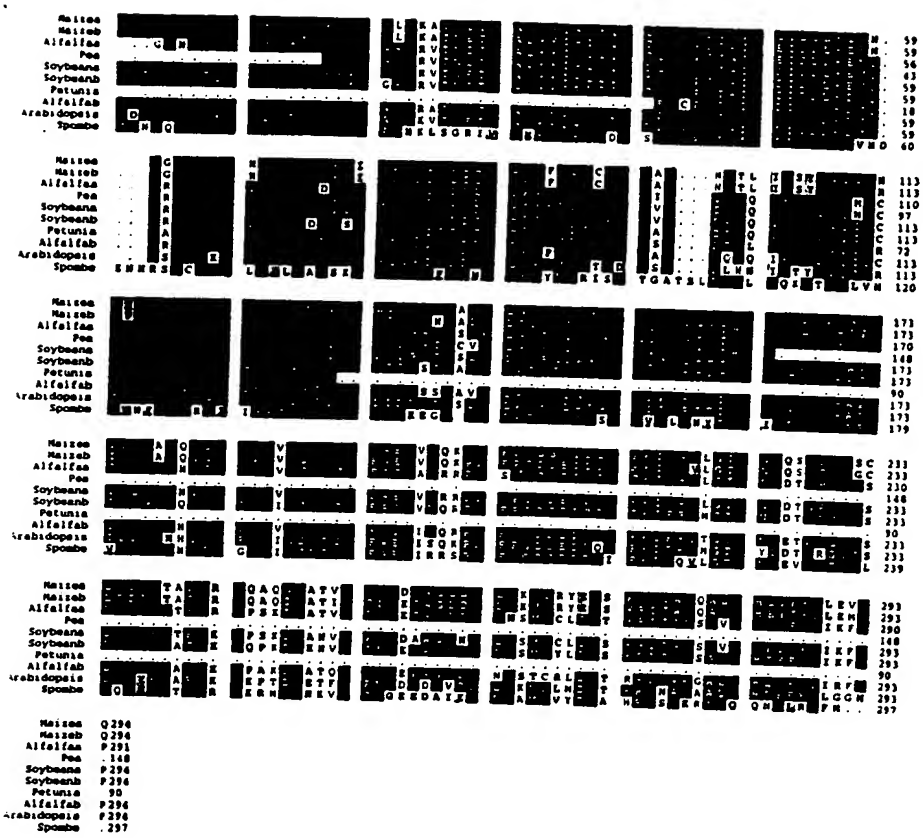

Fig. 1. PILEUP (Genetics Computer Group 1991) alignment of sequences of homologues of *cdc2* from maize (Colasanti et al. 1991), alfalfa (Hirt et al. 1991, 1993), pea (Feiler and Jacobs 1991), soybean (Miao et al. 1993), petunia (Bergounioux et al. 1992), *Arabidopsis* (Ferreira et al. 1991) and *Schizosaccharomyces pombe* (Hindley and Phear 1984).

fusion protein containing the carboxy-terminal region of human $p34^{cdc2}$ and to whole $p34^{cdc2}$ from fission yeast. A 34 kDa protein was recognised by these antibodies in *Chlamydomonas*, *Avena* and *Arabidopsis*. In contrast to yeast, the level of *Chlamydomonas* p34 varies, although both are phosphorylated in a cell cycle-dependent manner (John et al. 1989).

A monoclonal antibody raised against fission yeast p34 was also used to detect several related proteins in a range of higher plant species, together with a *cdc2*-related PCR product encoding 60% of the pea *cdc2* homologue (Feiler and Jacobs 1990). *cdc2* Homologues have since been isolated from *Arabidopsis* (Ferreira et al. 1991), *Zea mays* (Colasanti et al. 1991), *Medicago sativa* (alfalfa) (Hirt et al. 1991), *Petunia* (Bergounioux et al. 1992) and *Glycine max* (Miao et al. 1993). Despite the evolutionary divergence of these species, an alignment of their sequences shows an extraordinary degree of conservation (Fig. 1); a plot of their relative similarities, and their similarities with yeast and animal homologues, is shown in Fig. 2.

Compared with fission yeast, a higher level of complexity in human and *Xenopus* cells exists through the presence of two classes of *cdc2* homologue (Elledge and Spotswood 1991, Fang and Newport 1991). One functions at the G2–M transition and the other at the G1-S transition (*CDK2* in humans, *Eg1* in *Xenopus*) which suggests that the G1-S and G2-M transitions are controlled by different *cdc2* proteins in all higher eukaryotes. However, the data from plants on this issue are less conclusive. Alfalfa contains two *cdc2*-related genes, both present as single copies (Hirt et al. 1993). One (alfalfaa in Figs 1 and 2) complements the G2-M but not the G1-S block of budding yeast *cdc28ts* mutants whereas the other (alfalfab) complements the G1-S but not the G2-M block (Hirt et al. 1993). Hence, this would appear to mirror the situation in human cells and *Xenopus*. However, analysis of the primary sequence data shows that the alfalfab sequence is more closely related to other plant homologues, particularly alfalfaa and the pea homologue, than to *CDK2* or *Eg1*. Furthermore, only one *cdc2* gene has been isolated from pea and one from *Arabidopsis*; they are reported to be single copy (Feiler and Jacobs 1990, Ferreira et al. 1991). The situation is complicated further by the presence of '*cdc2*-like' genes in these species (Feiler and Jacobs 1991, Hirayama et al. 1991, Imajuku et al. 1992). These are undoubtedly related to *cdc2* but they lack a conserved PSTAIRE motif and fail to complement *cdc2* mutants of fission yeast (see Fig. 3).

Cell division in higher plants occurs in a multicellular environment with inevitable cell-cell interactions and cellular specialisations. Hence, we may expect greater levels of complexity in the organisation and regulation of plant *cdc2* homologues over and above that seen in yeasts and

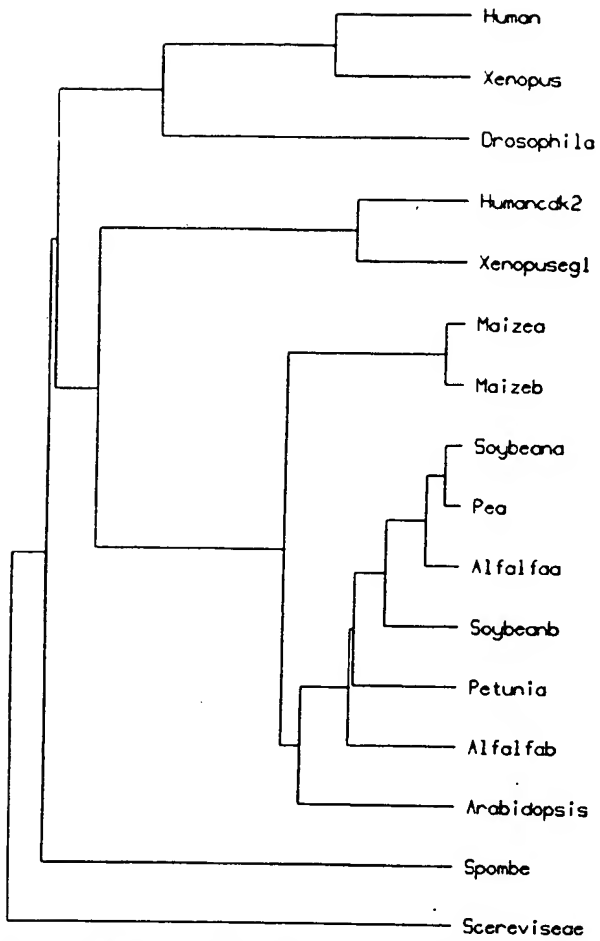

Fig. 2. Tree diagram generated from a PILEUP alignment of sequences of homologues of *cdc2* from human (Lee and Nurse 1987, Elledge and Spotswood 1991 [*cdk2*]), *Xenopus* (Pickham et al. 1992, Paris et al. 1991 [*Eg1*]), *Drosophila* (Jimenez et al. 1990, Lehner and O'Farrell 1990), maize (Colasanti et al. 1991), alfalfa (Hirt et al. 1991, 1993) pea (Feiler and Jacobs 1991), soybean (Miao et al. 1993), petunia (Bergounioux et al. 1992), *Arabidopsis* (Ferreira et al. 1991), *Schizosaccharomyces pombe* (Hindley and Phear 1984) and *Saccharomyces cerevisiae* (Lörincz and Reed 1984).

unicellular plants such as *Chlamydomonas*. One example of this is the variation in expression levels of *cdc2* between different plant organs. *cdc2* Transcripts in *Arabidopsis* show the highest levels of accumulation in leaf primordia, the vegetative shoot meristem, the basal regions of developing flower organs, the pericycle and the root tip meristem, i.e. areas with greater frequencies of, or potential for, cell division (Martinez et al. 1992). Similarly, in maize, $p34^{cdc2}$ is localised in meristematic zones such as the root apex (Colasanti et al. 1993). In soybean, expression of the different *cdc2* genes is differentially regulated. One is expressed more highly in roots and root nodules, and its expression is increased in response to *Rhizobium* infection. The other is more highly expressed in aerial tissues and does not respond to *Rhizobium* infection (Miao et al. 1993).

Cyclins

Cyclins are the second component of M-phase kinase. They were first identified in invertebrates as a result of their periodic accumulation during the cell cycle and rapid degradation at the end of mitosis (Evans et al. 1983). There are three distinct classes, based on primary sequence comparisons and expression patterns. These are termed cyclin A, cyclin B and G1 cyclins. The first to be sequenced was a cyclin A from clam; it is involved in the G2-M transition and can promote entry into meiosis when micro-injected into *Xenopus* oocytes (Swenson et al. 1986). Homologues have since been isolated from several species, including *Xenopus* (Minshull et al. 1989) and man (Wang et al. 1990). Cyclin B has been isolated from *Xenopus* (Minshull et al. 1990), man (Pines and Hunter 1989), clam (Westendorf et al. 1989) and fission yeast (Booher and Beach 1988, Hagan et al. 1988). It also promotes maturation of *Xenopus* oocytes (Solomon et al. 1990). Cyclins A and B show slightly different patterns of accumulation and degradation in the cell cycle (Minshull et al. 1990), but functional differences have yet to be fully established. Both are destroyed at the end of mitosis resulting in the loss of $p34^{cdc2}$ kinase activity (Murray et al. 1989). The G1 cyclins, such as the CLNs from *Saccharomyces cerevisiae*, are required for the G1-S phase transition (Hadwiger et al. 1989). Nasmyth and Hunt (1993) describe the oscillation of cyclins throughout interphase as a series of dams and sluices which regulate the cell through a multitude of checkpoints.

In plants, cyclins are generally less well conserved than *cdc2* homologues, but there is sufficient sequence similarity for a number of plant homologues to have been cloned using degenerate oligonucleotides as probes, or using PCR primers. Hata et al. (1991) isolated a carrot cyclin and two soybean cyclins by probing cDNA libraries with degenerate oligonucleotides. As in other systems, they are expressed at much higher levels in dividing tissues such as carrot cells undergoing somatic embryogenesis, or developing soybean leaves and roots, than in non-dividing tissues such as the internodes of stems

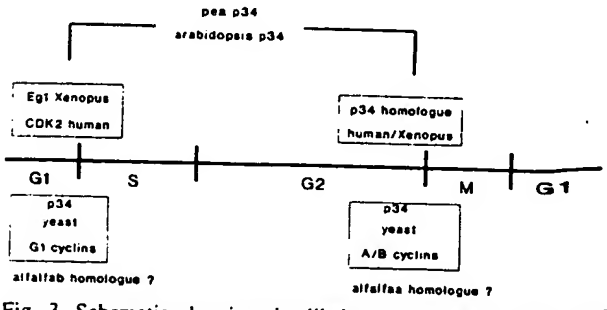

Fig. 3. Schematic showing the likely temporal expression of *cdc2* genes in the cell cycle. See text for explanations.

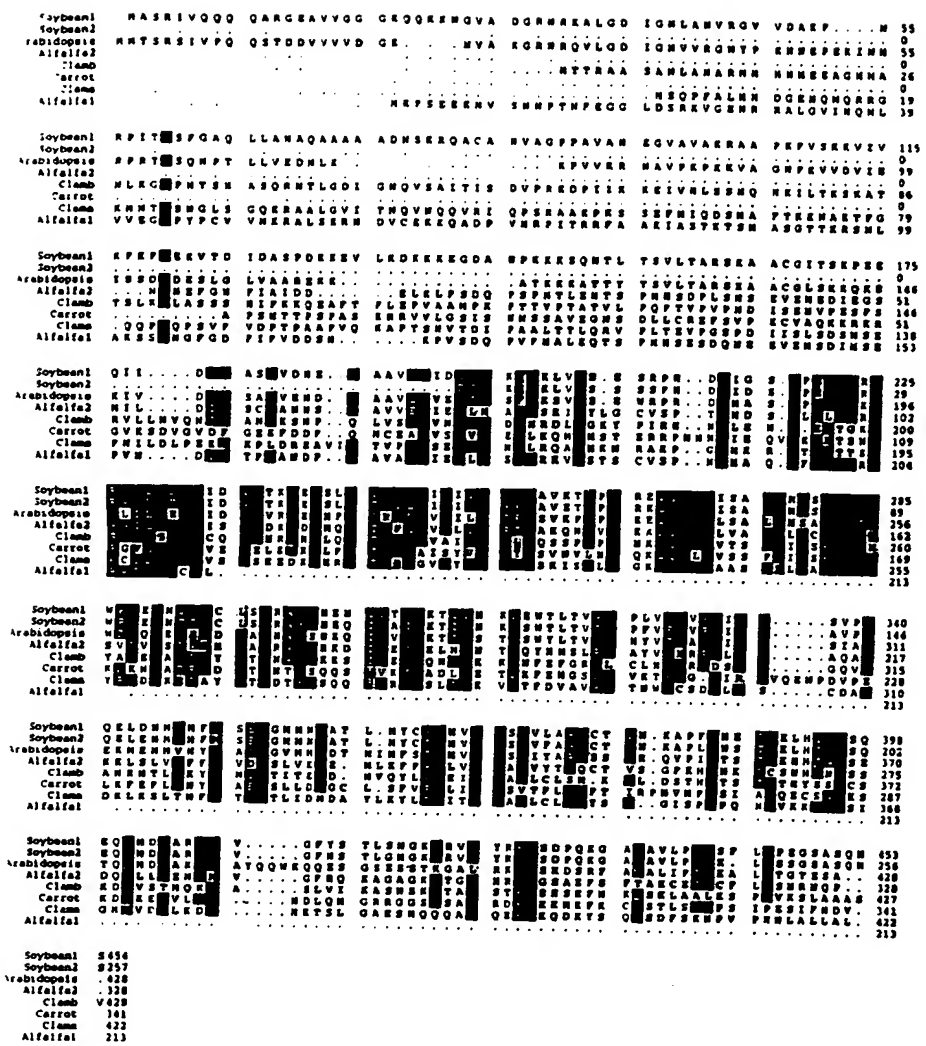

Fig. 4. PILEUP (Genetics Computer Group 1991) alignment of cyclin sequences from soybean and carrot (Hata et al. 1991), *Arabidopsis* (Hemerley et al. 1992) and alfalfa (Hirt et al. 1992), with clam cyclin A (Clama, Swenson et al. 1986) and clam cyclin B (Clamb, Westendorf et al. 1989).

(Hata et al. 1991). Hemerly et al. (1992) used degenerate oligonucleotides to amplify a cyclin sequence from *Arabidopsis*. Again, its mRNA accumulated in actively dividing tissues but was undetectable following treatment with hydroxyurea or colchicine which block S and M phases, respectively. Finally, Hirt et al. (1992) isolated from alfalfa two cyclin sequences whose expression was cell cycle-dependent.

Whether or not plant cyclins belong to class A, B or to the CLN cyclins is unclear. The conserved regions of the plant cyclins are aligned with clam A- and B-type cyclins in Fig. 4, and a similarity plot of the cyclin family is shown in Fig. 5. The carrot cyclin is a little more similar to class A cyclins than to the others, but has some class B characteristics. Conversely, the two soybean cyclins, the *Arabidopsis* cyclin and one of the alfalfa cyclins are closer to the B class, but have some A-class characteristics. The second alfalfa homologue cannot be classified at all on the basis of its sequence data.

Other plant *cdc*s?

To date, full length plant homologues to genes which regulate *cdc2* (e.g. *cdc25*, *wee1* and *nim1*) have yet to be isolated. Why the failures? These genes, and their gene products, are typically expressed at transient points in the cell cycle. For example, in fission yeast, $p80^{cdc25}$ peaks transiently in late G2 while the level of p34 remains fairly constant (Russell and Nurse 1986). In *Drosophila*, string was identified from a system exhibiting 100% synchrony of nuclear division in a coenocytic stage of development (Edgar and O'Farrell 1989); this system was probably enriched for p80. Plant meristems, which have served well for the isolation of plant homologues to *cdc2*, typically comprise asynchronous populations of cells and, hence, the levels of mRNA transcribed from these regulatory genes are probably very low.

Although *cdc2* and cyclin homologues exist in a range of unrelated plant species much needs to be learnt about the regulation of these genes and how they function

need to know is the underlying mechanism(s) which regulate the positioning of ppbs.

Plant cells with their unique structures may well house a substrate(s) for p34 kinase which is different from that in animal cells. Hence, it becomes useful to know about proteins that are associated with p34 and which are known to be functional during the transition from G2 to mitosis, particularly those involved with, or bound to, mitotic microtubules. One such group of proteins is the microtubule-associated proteins (MAPs) and another, is the mitogen-activated protein kinases (MAP kinases) which play a variety of roles in signal transduction but can also phosphorylate MAPs. In *Xenopus*, injection of MPF stimulates MAP kinase, probably as an up-stream activator of p34 kinase (Gotoh et al. 1991). Given the phosphorylation cascade which drives cells through the various check-points of the cell cycle, protein kinases associated with the mitotic microtubules are of special interest. While *cdc2* is turned off by phosphorylation on Tyr-15, MAP kinase is turned on by phosphorylation (Payne et al. 1991); Hunt (1991) suggested that the latter phosphorylation occurs at a tyrosine residue analogous in position to Thr161 of p34. If so, this would be remarkably similar to the phosphorylation of this residue in p34 prior to binding with cyclin B. A plant homologue to MAP kinases has been isolated from alfalfa. A cDNA clone showed 52% identity to rat ERK1, but also homology to the different homologues of the fission yeast *cdc2* kinase (30–40%); MAP kinases are part of a superfamily of *cdc2* kinases (Jonak et al. 1993). These authors suggest that the plant MAP kinase may be involved in responses to plant growth regulators in the way that animal MAP kinases participate in signal transduction pathways. Similarly, a full-length cDNA clone encoding a MAP kinase has also been isolated from alfalfa (Duerr et al. 1993). This MAP kinase, christened MsERK1 and recognised by antibodies against MAP kinases from three animal sources, may have a role in mitogenic induction in symbiotic root nodules of alfalfa (Duerr et al. 1993).

Cell size

During vegetative growth, pattern formation manifests itself as an iterative program in which leaves and axillary buds develop above-ground and roots below-ground. Cell division is an essential component of the program. For example, cell division occurs in shoot meristems so that new cells are invested into leaf primordia when the apex becomes partitioned at the end of a plastochron. Thus, new cells are the units for subsequent differentiated form and the cell cycle is the 'cell factory', but does it simply manufacture identical units which are shaped and patterned elsewhere? It would follow that meristems comprise homogenous populations of cells. However, this is far from being true. Characteristic features of meristems is the mosaic of cells it contains, the heterogeneity of cell cycles and the variable cell sizes therein. In the root meristem, this heterogeneity is reflected in a number of tissue systems: stelar, cortical and epidermal, all of which arise from apical initials. Not only is there a heterogeneity because of tissue differences but also cells get larger with increasing distance from the apex (Barlow 1971). Indeed, Barlow (1971) concluded that changes in cell size have an important bearing on the regulation of cell division and differentiation. According to this model, cell growth in more basal cells outpaces the synthetic rates necessary for division. However, increases in cell size do not occur randomly but are tightly regulated as cells are displaced from the meristem within a tissue domain (Barlow 1971, John et al. 1993).

Hunt (1991) regarded a regular sequence of cell growth and division (Curtis 1983) as an ideal definition of the cell cycle. John et al. (1993) noted that the attainment of a minimum critical cell size is an essential determinant for cell division with the major check-points occurring in late G1 (start) and late G2 (see above). While not wishing to deny the importance of *cdc2* and its positive and negative regulators, the cell growth cycle is probably regulated at many steps during interphase so that multiple checkpoints ensure that the cell divides normally. In other words, cell size is constantly monitored. John et al. (1993) in commenting on the localisation of p34 in relation to cell size at division, drew two highly pertinent conclusions. First, the cell cycle stabilises cell size. Second, size at division is altered as cells are displaced to the periphery of the meristem even though the size of the initials of different cell types (tissues) are different. Barlow's (1971) model is also highly relevant here. Cell size at division varies from tissue to tissue but the programming of cells for differentiation is set when the cells are meristematic. This concurs with an idea of Van't Hof's (1973) that cells are programmed to differentiate whilst meristematic. In other words, on a tissue-specific basis, a minimum size control regulates cell size at division and probably regulates at which point (or size) a cell goes out of cycle once displaced from the meristematic domain.

Various observations all point to a cell size control being an important component of the control of differentiation in higher plants. So, it seems that the cell cycle is not only the mechanism which produces cells but, through its major check-points, can regulate cell size at division and, hence, can impose a size control for differentiated cells in different tissues. We tested this model by deliberately perturbing cell size. Tobacco plants were transformed with fission yeast *cdc25* under the control of the CaMV 35S gene promoter. The transgenic plants showed clear evidence of expression of the fission yeast gene and exhibited various perturbations of their development compared with the wild type. The plants showed pocketing in the leaves, precocious flowering, more flowers per flowering head and petalless flowers alongside normal ones (Bell et al. 1993). To date, our analyses have shown that cells within the meristem of secondary roots are smaller at birth in the 35S–*cdc25* plants compared with the wild-type and it is probable that each of these abnormal phenotypes occur at least partly

because of perturbation of cell size at division in particular tissue systems. For example, in one of the few studies on the pattern of cell division during floral morphogenesis, rates of division fluctuated from slower to faster to slower during successive whorl formation (Lyndon and Cunninghame 1987). Petalless aberrant flowers in the *cdc25*-transgenic tobacco plants may have arisen because the normal timing of cell division, oscillating between slower and faster cell cycles, was disrupted by *cdc25* causing cells to divide prematurely. In other words, the perturbation may have specifically altered the timing of cell division in different floral domains so that the aberrant flowers lacked the petal whorl. Why only one whorl was affected in this way is unknown. In *Arabidopsis*, a gene which regulates floral meristem structure, *CLAVATA1*, may do so by regulating cell division patterns (Clark et al. 1993). It should prove fascinating to discover the extent to which this gene, classified by Weigel and Meyerowitz (1993) as cadastral, and the genetic hierarchy of meristem identity genes and homoetic genes, interact with regulatory *cdc* genes during floral morphogenesis.

Conclusions

(1) Searching for plant homologues to yeast *cdc* genes is of course essential. Equally clear is that their isolation will not provide the whole story.

(2) Although it is now established that in animal cells different *cdc2* genes regulate the G1 to S phase and G2 to M transitions, the situation in plants remains unclear. The assumption that a similar system operates in both plant and animal cells may prove incorrect. This may, in part, reflect different types of control on cell division in plants and animals.

(3) Clearly, there are structural differences between mitosis in animals and higher plants, with centrioles present in the former but absent in the latter. Moreover, whereas p34 can bind to mitotic microtubules and centrioles of animals cells (Bailly et al. 1989), it binds to preprophase bands (ppb) in plant cells (although not exclusively; Mineyuki et al. 1991, Colasanti et al. 1993, John et al. 1993). These differences suggest that at the G2-M transition, either p34 has different natural substrates in plants and animals or the substrates are the same but have different cellular locations. Hence, it is not too surprising to discover differences between *cdc2*s in plants and animals although, the modes of action of the *cdc2*s and the cyclins in plants still await a full explanation.

(4) The ppb is central to predicting planes of cell division in plant cells and p34 has been shown to bind to ppbs. However, we need to know how the ppbs are positioned and how gene products that regulate mitotic microtubule orientation interact with the *cdc* kinases. In this respect, understanding the role of MAPs and MAP kinases will be crucial.

(5) In biochemical terms, the cell cycle is a phosphorylation cascade which enables cells to enter mitosis. In structural terms, the cell cycle represents a series of check-points which ensure that cell size is optimal for division. Constitutive expression of the mitotic inducer gene *cdc25* from fission yeast in tobacco, perturbs various aspects of development and results in reduced cell size in the transgenic plants. It seems that *cdc25* can alter the cell cycle with consequent effects on cell size and on development.

(6) Through its multitude of check-points, the cell cycle not only comprises the sequence of events which produces more cells, but probably also imparts a size control having an important influence on cell division and development. Understanding the molecular regulation of cell size, which in turn will require a clear explanation of the function of plant *cdc* genes, should provide a clearer understanding of the relationship between the cell cycle and plant development.

References

Aves, S.J. & Bryant, J.A. 1993. The molecular genetics and biochemistry of DNA replication. – *In* Molecular and Cell Biology of the Plant Cell Cycle (J.C. Ormrod and D. Francis, eds), pp. 45–46. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-1767-X.

Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I. Nurse, P. & Glover, D. 1992. twine, A *cdc25* homologue with functions in the male and female germline of *Drosophila*. – Cell 69: 977–988.

Bailly, E., Dorée, M., Nurse, P. & Bornens, M. 1989. $p34^{cdc2}$ Is located in both nucleus and cytoplasm; part is centrosomally associated at G2/M and enters vesicles at anaphase. – EMBO J. 8: 3985–3995.

Barlow, P.W. 1971. Properties of cells in the root apex. – Rev. Fac. Agron. Univ. Nac. La Plata 47: 275–301.

Bell, M.H., Halford, N.G., Ormrod, J.C. & Francis, D. 1993. Tobacco plants transformed with *cdc25*, a mitotic inducer gene from fission yeast. – Plant Mol. Biol. 23: 445–451.

Bergounioux, C., Perennes, C., Hemerly, A.S., Qin, L.X., Sarda, C., Inze, D. & Gadal, P. 1992. A *cdc2* gene of *Petunia hybrida* is differentially expressed in leaves, protoplasts and during various cell cycle phases. – Plant Mol. Biol. 20: 1121–1130.

Booher, R. & Beach, D. 1988. Involvement of *cdc13*$^+$ in mitotic control in *S. pombe*: possible interaction of the gene with microtubules. – EMBO J. 7: 2321–2327.

Clark, S.E., Running, M.P. & Meyerowitz, E.M. 1993. *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. – Development 119: 397–418.

Colasanti, J., Tyers, M. & Sunderesan, V. 1991. Isolation and characterisation of cDNA clones encoding a functional $p34^{cdc2}$ homologue from *Zea mays*. – Proc. Natl. Acad. Sci. USA 88: 3377–3381.

– , Cho, S.-O., Wick, S. & Sunderesan, V. 1993. Localisation of the functional $p34^{cdc2}$ homolog of maize in root tip and stomatal complex cells: Association with predicted division sites. – Plant Cell 5: 1101–1111.

Curtis, H., ed. 1983. Biology, 4th Edition. Worth, New York, NY. ISBN 0-8790-1186-6.

Davidson, D. 1991. Cell division. – *In* Plant Physiology A

Treatise, Vol X: Growth and Development (F.C. Steward, ed.), pp. 341–436. Academic Press, New York, NY. ISBN 0-1266-8610-6.

Duerr, B., Gawienowski, M., Ropp, T. & Jacobs, T. 1993. MsERK1: a mitogen-activated protein kinase from a flowering plant. – Plant Cell 5: 87–96.

Edgar, B.A. & O'Farrell, P.H. 1989. Genetic control of cell division patterns in the *Drosophila* embryo. – Cell 57: 177–187.

– & O'Farrell, P.H. 1990. The three post blastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by string. – Cell 62: 469–480.

Eleftheriou, E.P. & Palevitz, B.A. 1992. The effect of cytochalasin D on preprophase band organisation in root tip cells of *Allium*. – J. Cell Sci. 103: 989–998.

Elledge S.J. & Spottswood, M.R. 1991. A human p34 protein kinase, *CDK2*, identified by complementation of a *cdc28* mutation in *Saccharomyces cerevisiae*, is a homolog of *Xenopus Eg1*. – EMBO J. 10: 2653–2659.

Evans, T., Rosenthall, E., Youngbloom, J., Distel, D. & Hunt, T. 1983. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. – Cell 33: 389–396.

Fang, F. & Newport, J.W. 1991. Evidence that the G1-S and G2-M transitions are controlled by different *cdc2* proteins in higher eukaryotes. – Cell 66: 731–742.

Feiler, H. S. & Jacobs, T. W. 1990. Cell division in higher plants: A *cdc2* gene, its 34-kDa product, and histone H1 kinase activity in pea. – Proc. Natl. Acad. Sci. USA 87: 5397–5401.

– & Jacobs, T.W. 1991. Cloning of the pea *cdc2* homologue by efficient immunological screening of PCR products. – Plant Mol. Biol. 17: 321–333.

Ferreira, P.C.G., Hemerly, A.S., Villaroel, R., Van Montagu, M. & Inze, D. 1991. The *Arabidopsis* functional homologue of the $p34^{cdc2}$ protein kinase. – Plant Cell 3: 531–540.

Francis, D. 1992. The cell cycle in plant development. – New Phytol. 122: 1–20.

Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T. & Maller, J.L. 1990. Cyclin is a component of MPF from *Xenopus*. – Cell 60: 487–494.

Genetics Computer Group. 1991. Program Manual for the GCG Package, Version 7, April 1991. – GCG, Madison, WI.

Gotoh, Y., Moriyama, S., Matsuda, E., Okomura, T., Kishimoto, H., Kawasaki, K., Suzuki, I., Yahara, H., Sakai, H. & Nishida, E. 1991. *Xenopus* M phase MAP kinase: Isolation of its cDNA and activation by MPF. – EMBO J. 10: 2661–2668.

Gunning, B.E.S. & Wick, S.M. 1985. Preprophase bands, phragmoplasts, and spatial control of cytokinesis. – J. Cell Sci. (Suppl.) 2: 157–179.

Hadwiger, J.A., Wittenberg, C., Richardson, H.E., de Barros Lopes, M. & Reed, S.I. 1989. A family of cyclin homologs that control the G1 phase in yeast. – Proc. Natl. Acad. Sci. USA 86: 6255–6259.

Hagana, I., Hayles, J. & Nurse, P. 1988. Cloning and sequencing of the cyclin-related *CDC13*$^+$ gene and a cytological study of its role in fission yeast mitosis. – J. Cell Sci. 91: 587–595.

Hata, S., Kouchi, H., Suzuka, I. & Ishii, T. 1991. Isolation and characterisation of cDNA clones for plant cyclins. – EMBO J. 10: 2681–2688.

Hayles, J. & Nurse, P. 1993. The controls acting at mitosis in *Schizosaccharomyces pombe*. – *In* Molecular and Cell Biology of the Plant Cell Cycle (J.C. Ormrod and D. Francis, eds), pp. 1–7. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-1767-X.

Hemerly, A., Bergounioux, C., Van Montagu, M., Inzé, D. & Ferreira, P. 1992. Genes regulating the plant cell cycle: Isolation of mitotic-like cyclin from *Arabidopsis thaliana*. – Proc. Natl. Acad. Sci. USA 89: 3295–3299.

Hindley, J. & Phear, G.A. 1984. Sequence of the cell division gene CDC2 for *Saccharomyces pombe*: patterns of splicing and homology to protein kinases. – Gene 31: 129–134.

Hirayama, T., Imajuku, Y., Anai, T., Matsui, M. & Oka, A. 1991. Identification of 2 cell-cycle-controlling *cdc2* gene homologs in *Arabidopsis thaliana*. – Gene 105: 159–165.

Hirt, H., Pay, A., Györgyey, J., Bako, L., Nemeth, K., Bögre, L., Schweyen, R.J., Herbele-Bors, E. & Dudits, D. 1991. Complementation of a yeast cell cycle mutant by an alfalfa cDNA encoding a protein kinase homologous to $p34^{cdc2}$. – Proc. Natl. Acad. Sci. USA 88: 1636–1640.

–, Mink, M., Pfosser, M., Bögre, L., Györgyey, J., Jonak, C., Gartner, A., Dudits, D. & Heberle-Bors, E. 1992. Alfalfa cyclins: differential expression during the cell cycle and in plant organs. – Plant Cell 4: 1531–1538.

–, Páy, A., Bögre, L., Meskiene, I. & Heberle-Bors, E. 1993. *cdc2MsB*, a cognate *cdc2* gene from alfalfa complements the G1/S but not the G2/M transition of budding yeast *cdc28* mutants. – Plant J. 4: 61–69.

Hunt, T. 1991. Summary: Put out more flags. – Cold Spring Harbor Symp. Quant. Biol. 56: 757–769.

Imajuku, Y., Hirayama, T., Endoh, H. & Oka, A. 1992. Exon-intron organisation of the *Arabidopsis thaliana* protein kinase genes *CDC2a* and *CDC2b*. – FEBS Lett. 304: 73–77.

Jacobs, T.J. 1992. Control of the cell cycle. – Develop. Biol. 153: 1–15.

Jimenez, J., Alphey, L., Nurse, P. & Glover, D.M. 1990. Complementation of fission yeast $cdc2^{ts}$ and $cdc25^{ts}$ mutants identifies two cell cycle genes from *Drosophila*: a *cdc2* homologue and string. – EMBO J. 9: 3565–3571.

John, P.C.L., Sek, F.J. & Lee, M.G. 1989. A homolog of the cell cycle control protein $p34^{cdc2}$ participates in the division cycle of *Chlamydomonas*, and a similar protein is detectable in higher plants and remote taxa. – Plant Cell 1: 1185–1193.

–, Zhang, K. & Dong, C. 1993. A $p34^{cdc2}$-based cell cycle: its significance in monocotyledenous, dicotyledonous and unicellular plants. – *In* Molecular and Cell Biology of the Plant Cell Cycle (J.C. Ormrod and D. Francis, eds), pp. 9–34. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-1767-X.

Jonak, C., Páy, A., Bögre, L., Hirt, H. & Heberle-Bors, E. 1993. The plant homologue of MAP kinase is expressed in a cell cycle-dependent and organ-specific manner. – Plant J. 3: 611–617.

Kumugai, A. & Dunphy, W.G. 1991. The *cdc25* protein controls tyrosine dephosphorylation of the *cdc2* protein in a cell-free system. – Cell 64: 903–914.

Krek, W. & Nigg, E.A. 1991. Differential phosphorylation of vertebrate $p34^{cdc2}$ kinase at the G1/S and G2/M transitions of the cell cycle: identification of major phosphorylation sites. – EMBO J. 10: 305–316.

Labbé, J.C., Capony, J.-P., Caput, D., Cavadore, J.-C., Derancourt, J., Kaghad, M., Lelias, J.-M., Picard, A. & Dorée, M. MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of *cdc2* and one molecule of cyclin B. – EMBO J. 8: 3053–3058.

Lee, M.G. & Nurse, P. 1987. Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. – Nature 327: 31–35.

Lehner, C.F. & O'Farrell, P.H. 1990. *Drosophila cdc2* homologues: a functional homolog is coexpressed with a cognate variant. – EMBO J. 9: 3573–3581.

Lörincz, A.T. & Reed, S.I. 1984. Primary structural homolgy between the product of yeast cell division control gene *cdc28* and vertebrate oncogenes. – Nature 307: 183–184.

Lundgren, K., Walworth, N., Booher, R., Dembski, M., Kirschner, M. & Beach, D. 1991. *mik1* and *wee1* cooperate in the inhibitory tyrosine phosphorylation of *cdc2*. – Cell 64: 1111–1122.

Lyndon, R.F. 1970. Planes of cell division and growth in the shoot apex of *Pisum*. – Ann. Bot. 34: 19–28.

–, Cunninghame, M.E. 1986. Control of shoot apical development via cell division. – *In* Plasticity in Plants, 40th Symp. Soc. Exp. Biol. (A.J. Trewavas and D.H. Jennings,

eds), pp. 233–255. Company of Biologists, Cambridge. ISBN 0-948601-03-5.
Martinez, M.C., Jørgenesen, J.-E., Lawton, M.A., Lamb, C.J. & Doerner, P.W. 1992. Spatial pattern of *cdc2* expression in relation to meristematic activity and cell proliferation during plant development. – Proc. Natl. Acad. Sci. USA. 89: 7360–7364.
McGowan, C.H. & Russell, P. 1993. Human *wee1* kinase inhibits cell division by phosphorylating $p34^{cdc2}$ exclusively on Tyr^{15}. – EMBO J. 12: 75–85.
Miao, G.-H., Hong, Z. & Verma, D.P.S. 1993. Two functional soybean genes encoding $p34^{cdc2}$ protein kinases are regulated by different plant developmental pathways. – Proc. Natl. Acad. Sci. USA 90: 943–947.
Mineyuki, Y., Yamashita, M. & Nagahama, Y. 1991. $p34^{cdc2}$ Kinase homologue in the preprophase band. – Protoplasma 162: 182–186.
Minshull, J., Blow, J.J. & Hunt, T. 1989. Translation of cyclin mRNA is necessary for extracts of activated *Xenopus* eggs to enter mitosis. – Cell 56: 947–956.
– , Golsteyn, R., Hill, C.S. & Hunt, T. 1990. The A- and B-type cyclin associated *cdc2* kinases in *Xenopus* turn on and turn off at different times in the cell cycle. – EMBO J. 9: 2865–2875.
Murray, A.W., Solomon, M.J. & Kirschner, M.W. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. – Nature 339: 280–283.
Nasmyth, K. & Hunt, T. 1993. Dams and sluices. – Nature 366: 634–635.
Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. – Nature 344: 503–508.
Palevitz, B.A. 1993. Morphological plasticity of the mitotic apparatus in plants and its developmental consequences. – Plant Cell 5: 1001–1009.
Paris, J., le Guellec, R., Couturier, A., le Guellec, K., Omilli, F., Camonis, J., MacNeill, S. & Philippe, M. 1991. Cloning by differential screening of a *Xenopus* cDNA coding for a protein highly homologous to *cdc2*. –Proc. Natl. Acad. USA 88: 1039–1043.
Parker, L.L., Atherton-Fessler, S., Lee, M.S., Ogg, S., Falk, J.L., Swenson, K.I. & Piwnica-Worms, H. 1991. Cyclin promotes the tyrosine phosphorylation of $p34^{cdc2}$ in a *wee1*$^+$ dependent manner. – EMBO J. 10: 1255–1263.
Payne, D., Rossomando, P., Martino, P., Erickson, A., Her, J., Shabanowitz, J., Hunt, D., Weber, M. & Sturgill, T. 1991. Identification of the regulatory phosphorylation sites in pp42/mitogen activated protein kinase (MAP kinase). – EMBO J. 10: 885–892.
Pickham, K.M., Meyer, A.N., Li, J. & Donoghue, D.J. 1992. Requirement of mosXE protein kinase for meiotic maturation of *Xenopus* oocytes induced by a *cdc2* mutant lacking regulatory phosphorylation sites. – Mol. Cell Biol. 12: 3192–3203.
Pines, J. & Hunter, T. 1989. Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with $p34^{cdc2}$. – Cell 58: 833–846.
Richardson, H.E., Stueland, C.S., Thomas, J., Russell, P. & Reed, S.I. 1990. Human cDNAs encoding homologs of the small $p34^{cdc28}$/^{cdc2}-associated protein of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. – Genes Dev. 4: 1332–1344.
Russell, P. & Nurse, P. 1986. *cdc25*$^+$ Functions as an inducer in the mitotic control of fission yeast. – Cell 45: 145–153.
– & Nurse, P. 1987a. Negative regulation of mitosis by *wee1*$^+$, a gene encoding a protein kinase homolog. – Cell 49: 559–567.
– & Nurse, P. 1987b. The mitotic inducer *nim1*$^+$ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. – Cell 49: 569–576.
Solomon, M.J., Booher, R., Kirschner, M. & Beach, D. 1988. Cyclin in fission yeast. – Cell 54: 738–739.
– , Glotzer, M., Lee, T.H., Phillippe, M. & Kirschner, M.W. 1990. Cyclin activation of $p34^{cdc2}$. – Cell 63: 1013–1024.
Strausfeld, U., Labbé, J.C., Fesquet, D., Cavadore, J.C., Picard, A., Sadhu, K., Russell, P. & Dorée, M. 1991. Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex in vitro by human CDC25 protein. – Nature 351: 242–244.
Swenson, K.I., Farrell, K.M. & Ruderman, J.V. 1986. The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. – Cell 47: 861–870.
Van't Hof, J. 1973. The regulation of cell division in higher plants. – Brook. Symp. 25: 152–165.
Wang, J., Chenivesse, X., Henglein, B. & Brechot, C. 1990. Hepatitis B virus integration in a cyclin-A gene in a hepatocellular carcinoma. – Nature 343: 555–557.
Weigel, D. & Meyerowitz, E.M. 1993. Genetic hierarchy controlling flower development. – *In* Molecular Basis of Morphogenesis, 51st Annu. Symp. Soc. Dev. Biol. (M. Bernfield, ed.), pp. 93–107. Wiley-Liss Inc., New York, NY. ISBN 0-4713-0515-4.
Westendorf, J.M., Swenson, K.I. & Ruderman, J.V. 1989. The role of cyclin in meiosis-I. – J. Cell. Biol. 108: 1431–1444.
Wetmore, R.H. & Sorokin, S. 1955. On the differentiation of xylem. – J. Arnold. Arb. 36: 305–317.
Whiteway, M., Dignard, D. & Thomas, D.Y. 1992. Dominant negative selection of heterologous genes: isolation of *Candida albicans* genes that interfere with *Saccharomyces cerevisiae* mating factor-induced cell cycle arrest. – Proc. Natl. Acad. Sci. USA 89: 9410–9414.
Yamashita, M., Fukada, S., Yoshikuni, M., Bulet, P., Hirai, T., Yamaguchi, A., Lou, Y.-H., Zhao, Z. & Nagahama, Y. 1992. Purification and characterisation of maturation promoting factor in fish. – Dev. Biol. 149: 8–15.

Edited by D. A. Tepfer